(FILE 'HOME' ENTERED AT 11:01:50 ON 21 MAR 2001)

FILE 'MEDLINE, EMBASE, SCISEARCH, USPATFULL' ENTERED AT 11:02:03 ON 21 MAR 2001

L1 401 S (FLOW (S) CYTOMET?) AND (HIGH THROUGHPUT)
L2 140 S (FLOW (S) CYTOMET?) (10P) (HIGH THROUGHPUT)
L3 91 S L2 AND (?SAMPL?)
L4 63 DUP REM L3 (28 DUPLICATES REMOVED)

L4 ANSWER 1 OF 63 USPATFULL
TI Halide indicators

L4 ANSWER 2 OF 63 USPATFULL
TI Human interferon-.epsilon.(IFN-.epsilon.), a type I interferon

L4 ANSWER 3 OF 63 USPATFULL
TI IP-10/Mig receptor designated CXCR3, antibodies, nucleic acids, and methods of use therefor

L4 ANSWER 4 OF 63 USPATFULL
TI Methods and compositions for use in modulating expression of matrix metalloproteinase genes

L4 ANSWER 5 OF 63 USPATFULL
TI High throughput screening for novel enzymes

L4 ANSWER 6 OF 63 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 1
TI Plug flow cytometry extends analytical capabilities in cell adhesion and receptor pharmacology.

L4 ANSWER 7 OF 63 USPATFULL
TI Reaction vessel agitation apparatus

L4 ANSWER 8 OF 63 USPATFULL
TI Polypeptides expressed in skin cells

L4 ANSWER 9 OF 63 USPATFULL
TI Method and characterizing polymer molecules or the like

L4 ANSWER 10 OF 63 USPATFULL
TI Therapeutic compositions and methods and diagnostic assays for type II diabetes involving HNF-1

L4 ANSWER 11 OF 63 USPATFULL
TI Fluid **sample** for analysis controlled by total fluid volume and by total particle counts

L4 ANSWER 12 OF 63 USPATFULL
TI Method of detecting or identifying ligands, inhibitors or promoters of CXC chemokine receptor 3

L4 ANSWER 13 OF 63 USPATFULL
TI Human intronic and polymorphic SR-BI nucleic acids and uses therefor

L4 ANSWER 14 OF 63 USPATFULL
TI Capillary assays involving separation of free and bound species

L4 ANSWER 15 OF 63 USPATFULL
TI Multiplexed molecular analysis apparatus and method

L4 ANSWER 16 OF 63 USPATFULL
TI Biologically active alternative form of the ikka.alpha. I.kappa.B kinase

L4 ANSWER 17 OF 63 USPATFULL
TI Glycoconjugates and methods

L4 ANSWER 18 OF 63 USPATFULL

TI Serotonin 5-HT6 receptor knockout mouse

L4 ANSWER 19 OF 63 USPATFULL
TI Apolipoprotein E transgenic mice and assay methods

L4 ANSWER 20 OF 63 USPATFULL
TI Modulation of mammalian telomerase by peptide nucleic acids

L4 ANSWER 21 OF 63 USPATFULL
TI Fluorogenic peptides for the detection of protease activity

L4 ANSWER 22 OF 63 USPATFULL
TI Patched genes and their use for diagnostics

L4 ANSWER 23 OF 63 USPATFULL
TI Highly sensitive, accurate, and precise automated method and device for identifying and quantifying platelets and for determining platelet activation state using whole blood **samples**

L4 ANSWER 24 OF 63 MEDLINE DUPLICATE 2
TI **Flow cytometric** analysis of immunoprecipitates: **high-throughput** analysis of protein phosphorylation and protein-protein interactions.

L4 ANSWER 25 OF 63 MEDLINE DUPLICATE 3
TI **Flow cytometry**-based minisequencing: a new platform for **high-throughput** single-nucleotide polymorphism scoring.

L4 ANSWER 26 OF 63 MEDLINE DUPLICATE 4
TI Multiplexed single nucleotide polymorphism genotyping by oligonucleotide ligation and flow cytometry.

L4 ANSWER 27 OF 63 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 5
TI Rapid identification of local T cell expansion in inflammatory organ diseases by flow cytometric T cell receptor V.beta. analysis.

L4 ANSWER 28 OF 63 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 6
TI Flow cytometric measurement of intracellular cytokines.

L4 ANSWER 29 OF 63 MEDLINE DUPLICATE 7
TI Enumeration of micronucleated reticulocytes in rat peripheral blood: a flow cytometric study.

L4 ANSWER 30 OF 63 MEDLINE DUPLICATE 8
TI **High-throughput flow cytometric** DNA fragment sizing.

L4 ANSWER 31 OF 63 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 9
TI Commercial high speed machines open new opportunities in **high throughput flow cytometry** (HTFC).

L4 ANSWER 32 OF 63 USPATFULL
TI Assay and kit for determining the condition of cells

L4 ANSWER 33 OF 63 USPATFULL
TI Trio molecules and uses related thereto

L4 ANSWER 34 OF 63 USPATFULL
TI Drug screening process

L4 ANSWER 35 OF 63 USPATFULL
TI CD44-like protein and nucleic acids

L4 ANSWER 36 OF 63 USPATFULL
TI Nucleic acids encoding tumor virus susceptibility genes

L4 ANSWER 37 OF 63 USPATFULL
TI Drug screening process measuring changes in cell volume

L4 ANSWER 38 OF 63 USPATFULL
TI Assays and reagents for identifying modulators of cdc25-mediated mitotic
activation

L4 ANSWER 39 OF 63 USPATFULL
TI Apparatus and method for performing electrodynamic focusing on a microchip

L4 ANSWER 40 OF 63 MEDLINE DUPLICATE 10
TI A flow cytometric opsonophagocytic assay for measurement of functional antibodies elicited after vaccination with the 23-valent pneumococcal polysaccharide vaccine.

L4 ANSWER 41 OF 63 MEDLINE DUPLICATE 11
TI Photon-burst analysis in two-photon fluorescence excitation flow cytometry.

L4 ANSWER 42 OF 63 MEDLINE DUPLICATE 12
TI Plug flow cytometry: An automated coupling device for rapid sequential flow cytometric **sample** analysis.

L4 ANSWER 43 OF 63 MEDLINE DUPLICATE 13
TI Sheath fluid control to permit stable flow in rapid mix flow cytometry.

L4 ANSWER 44 OF 63 USPATFULL
TI Automated method and device for identifying and quantifying platelets and for determining platelet activation state using whole blood **samples**

L4 ANSWER 45 OF 63 USPATFULL
TI Methods for identifying compounds useful in treating type II diabetes

L4 ANSWER 46 OF 63 USPATFULL
TI Isolated nucleic acid encoding corticotropin-releasing factor.sub.2 receptors

L4 ANSWER 47 OF 63 USPATFULL
TI Simultaneous human ABO and RH(D) blood typing or antibody screening by flow cytometry

L4 ANSWER 48 OF 63 USPATFULL
TI Method for preparation and analysis of leukocytes in whole blood

L4 ANSWER 49 OF 63 USPATFULL
TI Methods for the rapid analysis of the reticulocytes

L4 ANSWER 50 OF 63 USPATFULL
TI Method for quantitatively measuring apoptosis

L4 ANSWER 51 OF 63 USPATFULL
TI Flow sorter with video-regulated droplet spacing

L4 ANSWER 52 OF 63 USPATFULL
TI Compositions and methods for the rapid analysis of reticulocytes

L4 ANSWER 53 OF 63 USPATFULL
TI Methods and apparatus for DNA sequencing

L4 ANSWER 54 OF 63 USPATFULL
TI Synthesizing and screening molecular diversity

L4 ANSWER 55 OF 63 MEDLINE DUPLICATE 14
TI Flow cytometric analysis of micronucleated reticulocytes in mouse bone marrow.

L4 ANSWER 56 OF 63 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 15
TI Simple and reliable enumeration of micronucleated reticulocytes with a single-laser flow cytometer.

L4 ANSWER 57 OF 63 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 16
TI General concepts about cell sorting techniques.

L4 ANSWER 58 OF 63 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 17
TI Development of a robust flow cytometric assay for determining numbers of viable bacteria.

L4 ANSWER 59 OF 63 USPATFULL
TI Apparatus and method for measuring fluorescence intensities at a plurality of wavelengths and lifetimes

L4 ANSWER 60 OF 63 USPATFULL
TI Parallel pulse processing and data acquisition for high speed, low error
flow cytometry

L4 ANSWER 61 OF 63 USPATFULL
TI Method and apparatus for simultaneously measuring a plurality of spectral wavelengths present in electromagnetic radiation

L4 ANSWER 62 OF 63 MEDLINE DUPLICATE 18
TI Fluorescence-based viability assay for studies of reactive drug intermediates.

L4 ANSWER 63 OF 63 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
TI A new flow cytometric transducer for fast **sample** throughput and time resolved kinetic studies of biological cells and other particles.

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L3 91 S L2 AND (?SAMPL?)
L4 63 DUP REM L3 (28 DUPLICATES REMOVED)

CCESSION NUMBER: 2001:7841 USPATFULL
TITLE: High throughput screening for novel enzymes
INVENTOR(S): Short, Jay M., Encinitas, CA, United States
Keller, Martin, San Diego, CA, United States
PATENT ASSIGNEE(S): Diversa Corporation, San Diego, CA, United States (U.S.
corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 6174673	20010116
APPLICATION INFO.:	US 1998-98206	19980616 (9)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1997-876276, filed on 16 Jun 1997
DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Yucel, Remy
LEGAL REPRESENTATIVE: Gray Gary Ware & Freidenrich LLP; Haile, Lisa A.
NUMBER OF CLAIMS: 23
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 18 Drawing Figure(s); 16 Drawing Page(s)
LINE COUNT: 2469
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

SUMM . . . Barns et. al 1994, Torvsik, 1990). For example, Norman Pace's laboratory recently reported intensive untapped diversity in water and sediment **samples** from the "Obsidian Pool" in Yellowstone National Park, a spring which has been studied since the early 1960's by microbiologists. . . Sargasso Sea while Torsvik et al. (1990) have shown by DNA reassociation kinetics that there is considerable diversity in soil **samples**. Hence, this vast majority of microorganisms represents an untapped resource for the discovery of novel biocatalysts.

In order to access. . .

SUMM . . . for example, 100's of different organisms requires the analysis of several million clones to cover this genomic diversity. An extremely **high-throughput** screening method has been developed to handle the enormous numbers of clones present in these libraries.

SUMM In traditional **flow cytometry**, it is common to analyze very large numbers of eukaryotic cells in a short period of time. Newly developed **flow cytometers** can analyze and sort up to 20,000 cells per second. In a typical **flow cytometer**, individual particles pass through an illumination zone and appropriate detectors, gated electronically, measure the magnitude of a pulse representing the. . . quantitative property versus the channel number (Davey and Kell, 1996). It was recognized early on that the data accruing from **flow cytometric** measurements could be analyzed (electronically) rapidly enough that electronic cell-sorting procedures could be used to sort cells with desired properties. . .

SUMM . . . the fluorescence can give quantitative data about specific target molecules or subcellular components and their distribution in the cell population. **Flow cytometry** can quantitate virtually any cell-associated property or cell organelle for which there is a fluorescent probe (or natural fluorescence). The. . .

SUMM **Flow cytometry** has also been used in cloning and selection of variants from existing cell clones. This selection,

however, has required stains. . . through cells passively, rapidly and irreversibly, with no toxic effects or other influences on metabolic
or physiological processes. Since, typically, **flow** sorting has been used to study animal cell culture performance, physiological state of cells, and the cell cycle, one goal. . .

SUMM A limited number of papers describing various applications of **flow cytometry** in the field of microbiology and
sorting of fluorescence activated microorganisms have, however, been published (Davey and Kell, 1996). Fluorescence. . . employed for microbial discrimination and identification, and in the analysis of the interaction of drugs and antibiotics with microbial cells. **Flow cytometry** has been used in aquatic biology, where
autofluorescence of photosynthetic pigments are used in the identification of algae or DNA stains are used to quantify and count marine populations (Davey and Kell, 1996). Thus, Diaper and Edwards used
flow cytometry to detect viable bacteria after
staining with a range of fluorogenic esters including fluorescein diacetate (FDA) derivatives and CemChrome B,. . .

SUMM Papers have also been published describing the application of **flow cytometry** to the detection of native and
recombinant enzymatic activities in eukaryotes. Betz et al. studied native (non-recombinant) lipase production by the eukaryote, Rhizopus arrhizus with **flow cytometry**. They found that spore
suspensions of the mold were heterogeneous as judged by light-scattering
data obtained with excitation at 633. . .

SUMM Scrienc et al. have reported a **flow cytometric**
method for detecting cloned -galactosidase activity in the eukaryotic organism, S. cerevisiae. The ability of **flow cytometry**
to make measurements on single cells means that individual cells with high levels of expression (e.g., due to gene amplification. . .

SUMM . . . sporulation loci in subtilis (spo). The technique used to monitor b-galactosidase expression from spo-lacZ fusions in single cells
involved taking **samples** from a sporulating culture, staining
them with a commercially available fluorogenic substrate for b-galactosidase called C8-FDG, and quantitatively analyzing fluorescence
in single cells by **flow cytometry**. In this study,
the **flow cytometer** was used as a detector to screen
for the presence of the spo gene during the development of the cells.. . .

SUMM Another group has utilized **flow cytometry** to
distinguish between the developmental stages of the delta-proteobacteria
Myxococcus xanthus (F. Russo-Marie, et.al., PNAS, Vol. 90, pp.8194-8198,
September 1993).. . .

SUMM . . . fluorogenic substrates, however, makes it possible to determine
.beta.-galactosidase activity in a large number of individual cells by means of **flow cytometry**. This type of determination
can be more informative with regard to the physiology of the cells, since gene expression can. . . compared the two molecules as substrates for .beta.-galactosidase, and concluded that FDG is a better substrate for .beta.-galactosidase detection by **flow cytometry** in bacterial cells. The screening performed in this
study was for the comparison of the two substrates. The detection capabilities. . .

SUMM Cells with chromogenic or fluorogenic substrates yield colored and fluorescent products, respectively. Previously, it had been thought that
the **flow cytometry**-fluorescence activated cell
sorter approaches could be of benefit only for the analysis of cells

that contain intracellularly, or are normally. . . could penetrate the cell and which are thus potentially cytotoxic. To avoid clumping of heterogeneous cells, it is desirable in **flow cytometry** to analyze only individual cells, and this could limit the sensitivity and therefore the concentration of target molecules that can. . . microdroplets may be made such that sufficient extracellular product remains associated with each individual gel microdroplet, so as to permit **flow cytometric** analysis and cell sorting on the basis of concentration of secreted molecule within each microdroplet. Beads have also been used. . .

SUMM The gel microdroplet technology has had significance in amplifying the signals available in **flow cytometric** analysis, and in permitting the screening of microbial strains in strain improvement programs for biotechnology. Wittrup et al., (Biotechnolo.Bioeng. (1993). . .

SUMM . . . or other related technologies can be used in the present invention to localize as well as amplify signals in the **high throughput** screening of recombinant libraries. Cell viability during the screening is not an issue or concern since nucleic acid can be. . .

SUMM The present invention adapts traditional eukaryotic **flow cytometry** cell sorting systems to **high throughput** screening for expression clones in prokaryotes. In the present invention, expression libraries derived from DNA, primarily DNA directly isolated from. . .

SUMM . . . or more expression libraries derived from nucleic acid directly isolated from the environment; and (ii) screening said libraries utilizing a **high throughput** cell analyzer, preferably a fluorescence activated cell sorter, to identify said clones.

SUMM . . . (ii) exposing said libraries to a particular substrate or substrates of interest; and (iii) screening said exposed libraries utilizing a **high throughput** cell analyzer, preferably a fluorescence activated cell sorter, to identify clones which react with the substrate or substrates.

SUMM . . . screening said exposed libraries utilizing an assay requiring a binding event or the covalent modification of a target, and a **high throughput** cell analyzer, preferably a fluorescence activated cell sorter, to identify positive clones.

SUMM . . . for target DNA sequences containing at least a partial coding region for at least one specified activity in a DNA **sample** by co-encapsulating a mixture of target DNA obtained from a mixture of organisms with a mixture of DNA probes including. . .

DETD The use of a culture-independent approach to directly clone genes encoding novel enzymes from environmental **samples** allows one to access untapped resources of biodiversity. The approach is based on the construction of "environmental libraries" which represent. . . cloning vectors that can be propagated in suitable prokaryotic hosts. Because the cloned DNA is initially extracted directly from environmental **samples**, the libraries are not limited to the small fraction of prokaryotes that can be grown in pure culture. Additionally, a normalization of the environmental DNA present in these **samples** could allow more equal representation of the DNA from all of the species present in the original **sample**. This can dramatically increase the efficiency of finding interesting genes from minor constituents of the **sample** which may be under-represented by several orders of magnitude compared to the dominant species.

DETD . . . the rapid screening of complex environmental expression libraries, containing, for example, thousands of different organisms. The analysis of a complex **sample** of this size requires one to screen several million clones to cover this genomic biodiversity. The invention represents an extremely **high-throughput**

screening method which allows one to assess this enormous number of clones. The method disclosed allows the screening anywhere from. . .

DETD The present invention combines a culture-independent approach to directly clone genes encoding novel bioactivities from environmental **samples** with an extremely **high throughput** screening system designed for the rapid discovery of new biomolecules.

DETD . . . collective genomes of naturally occurring microorganisms are generated. In this case, because the cloned DNA is extracted directly from environmental **samples**, the libraries are not limited to the small fraction of prokaryotes that can be grown in pure culture. In addition,. . . nucleic acid as one approach to more equally represent the DNA from all of the species present in the original **sample** . Normalization techniques can dramatically increase the efficiency of discovery from genomes which may represent minor constituents of the environmental **sample**. Normalization is preferable since at least one study has demonstrated that an organism of interest can be underrepresented by five. . .

DETD . . . Eubacteria and Archaebacteria, and lower eukaryotic microorganisms such as fungi, some algae and protozoa. Libraries may be produced from environmental **samples** in which case DNA may be recovered without culturing of an organism or the DNA may be recovered from a. . .

DETD . . . of microorganism DNA as a starting material library from which target DNA is obtained are particularly contemplated to include environmental **samples**, such as microbial **samples** obtained from Arctic and Antarctic ice, water or permafrost sources, materials of volcanic origin, materials from soil or plant sources. . .

DETD . . . the isolation of nucleic acid for generation of the expression gene library, FACS sorted to separate prokaryotic cells from the **sample** based on, for instance, DNA or AT/GC content of the cells. Various dyes or stains well known in the art, for example those described in "Practical **Flow Cytometry**", 1995 Wiley-Liss, Inc., Howard M. Shapiro, M.D., are used to intercalate or associate with nucleic acid of cells, and cells. . . content or AT/GC DNA content in the cells. Other criteria can also be used to separate prokaryotic cells from the **sample**, as well. DNA is then isolated from the cells and used for the generation of expression gene libraries, which are. . .

DETD . . . for isolation of activities of interest from a variety of sources, including consortias of microorganisms, primary enrichments, and environmental "uncultivated" **samples**, to make libraries which have been "normalized" in their representation of the genome populations in the original **samples**. and to screen these libraries for enzyme and other bioactivities. Libraries with equivalent representation of genomes from microbes that can. . .

DETD One embodiment for forming a normalized library from an environmental **sample** begins with the isolation of nucleic acid from the **sample**. This nucleic acid can then be fractionated prior to normalization to increase the chances of cloning DNA from minor species from the pool of organisms **sampled**. DNA can be fractionated using a density centrifugation technique, such as a cesium-chloride gradient. When an intercalating agent, such as. . . can be used to fractionate complex mixtures of genomes. This can be of particular value when working with complex environmental **samples**. Alternatively, the DNA does not have to be fractionated prior to normalization. **Samples** are recovered from the fractionated DNA, and the strands of nucleic acid are then melted and allowed to selectively reanneal. . .

DETD Hence, one embodiment for forming a normalized library from environmental **sample**(s) is by (a) isolating nucleic acid from the environmental **sample**(s); (b) optionally fractionating the

nucleic acid and recovering desired fractions; and (c) optionally normalizing the representation of the DNA within the population so as to

form a normalized expression library from the DNA of the environmental **sample**(s). The "normalization" process is described and exemplified in detail in co-pending, commonly assigned U.S. Ser. No. 08/665,565, filed Jun. 18,. . .

DETD The preparation of DNA from the **sample** is an important step in the generation of normalized or non-normalized DNA libraries from environmental **samples** composed of uncultivated organisms, or for the generation of libraries from cultivated organisms. DNA can be isolated from **samples** using various techniques well known in the art (Nucleic Acids in the Environment Methods & Applications, J. T. Trevors, D.. . . be of large size and free of enzyme inhibitors or other contaminants. DNA can be isolated directly from an environmental **sample** (direct lysis), or cells may be harvested from the **sample** prior to DNA recovery (cell separation). Direct lysis procedures have several advantages over protocols based on cell separation. The direct. . .

DETD Isolation of total genomic DNA from extreme environmental **samples** varies depending on the source and quantity of material. Uncontaminated, good quality (>20 kbp) DNA is required for the construction. . .

DETD Gene libraries can be generated by inserting the DNA isolated or derived from a **sample** into a vector or a plasmid. Such vectors or plasmids are preferably those containing expression regulatory sequences, including promoters, enhancers. . .

DETD . . . which is able to stably integrate large segments of genomic DNA. When integrated with DNA from a mixed uncultured environmental **sample**, this makes it possible to achieve large genomic fragments in the form of a stable "environmental DNA library."

DETD . . . during conjugation and is ideal to achieve and stably propagate large DNA fragments, such as gene clusters from mixed microbial **samples**. Other examples of vectors include cosmids, bacterial artificial chromosome vectors, and P1 vectors.

DETD . . . may have on their actions. Potential agent molecules are originally derived from a gene library generated from environmental or other **samples**, as described herein.

DETD . . . described (reviewed in Ihler, G. M. (1983) J. Pharm. Ther). These techniques are useful in the present invention to encapsulate **samples** for screening.

DETD . . . sequences are engineered between the two groups, and a hybridization event between the probe sequence and a target in a **sample** separates the protein from the quencher enough to yield a fluorescent signal. Still another group has reported a combination of. . .

DETD . . . of the cell, to molecules expressed by, or ultimately yielded by the expression of, members of gene libraries derived from **samples** and generated according to the methods described herein. For example, cellular events which can be detected with commercially available products. . .

DETD . . . discovery of novel aromatic compounds encoded by gene pathways, for example, encoded by polyketide genes, directly from environmental or other **samples**.

DETD Compounds can also be generated via the modification of host porphyrin-like molecules by gene products derived from these **samples**. Thus, one can screen for recombinant clone gene products which modify a host porphyrin-like compound to make it fluoresce.

DETD . . . as previously published, but to screen and recover positives in a manner that allows further screens to be performed on **samples**

selected. For example, typical stains used for enumeration can affect cell viability, therefore these types of stains were not employed. .

.

DETD The following outlines the procedures used to generate a gene library from an environmental **sample**.

DETD . . . minutes. After 30 minutes 31 .mu.l H.sub.2 O and 5 ml 10.times.

STE are added to the reaction and the **sample** is size fractionate on a Sephacryl S-500 spin column. The pooled fractions (1-3)

are phenol/chloroform extracted once followed by an. . . resulting pellet is washed with 1 ml 70% ethanol, repelleted by centrifugation and

allowed to dry for 10 minutes. The **sample** is resuspended in 10.5 .mu.l TE buffer. Do not plate. Instead, ligate directly to lambda arms as above except use. . .

DETD Sucrose Gradient (2.2 ml) Size Fractionation. Stop ligation by heating the **sample** to 65.degree. C. for 10 minutes. Gently load **sample** on 2.2 ml sucrose gradient and centrifuge in mini-ultracentrifuge at 45K, 20.degree. C. for 4 hours (no brake). Collect fractions. . .

DETD Test Ligation to Lambda Arms. Plate assay by spotting 0.5 .mu.l of the **sample** on agarose containing ethidium bromide along with standards (DNA **samples** of known concentration) to get an approximate concentration. View the **samples** using UV light and estimate concentration compared to the standards. Fraction 1-4=>1.0 .mu.g/.mu.l. Fraction 5-7=500 ng/.mu.l.

DETD

Sample	H.sub.2 O	10X Ligase Buffer	10 mM rATP	Lambda arms (ZAP)	Insert DNA	T4 DNA Ligase (4 Wu/(l)
Fraction 1-4	0.5 .mu.l	0.5 .mu.l	0.5 .mu.l	1.0 .mu.l	2.0 .mu.l	0.5 .mu.l

Fraction. . .

DETD Prior to library generation, purified DNA can be normalized. DNA is first fractionated according to the following protocol. A **sample** composed of genomic DNA is purified on a cesium-chloride gradient. The cesium chloride (Rf=1.3980) solution is filtered through a 0.2. . . ISCO UA-5 UV absorbance detector set to 280 nm. Peaks representing the DNA from the organisms present in an environmental **sample** are obtained. Eubacterial sequences can be detected by PCR amplification of DNA encoding rRNA from a 10-fold dilution of the. . .

DETD Normalization is then accomplished as follows by resuspending double-stranded DNA **sample** in hybridization buffer (0.12 M NaH.sub.2 PO.sub.4, pH 6.8/0.82 M NaCl/1 mM EDTA/0.1% SDS). The **sample** is overlaid with mineral oil and denatured by boiling for 10 minutes. **Sample** is incubated at 68.degree. C. for 12-36 hours. Double-stranded DNA is separated from single-stranded DNA according to standard protocols (Sambrook,. . .

DETD . . . H.sub.2 O, 1% DMSO, 1% EtOH) and 50 .mu.l Propidium iodide (PI)

staining solution (50 .mu.g/ml of distilled water). The **sample** is incubated in the dark at 37.degree. C. with shaking at 150rpm for 30 minutes. Cells are then heated to 70.degree. C. for 30 minutes (this step can be avoided if **sample** is not derived from a hyperthermophilic organism).

CLM What is claimed is:

13. The method of claim 1, wherein the **samples** are heated before step b).

L4 ANSWER 6 OF 63 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 1

ACCESSION NUMBER: 2001078253 EMBASE

TITLE: Plug flow cytometry extends analytical capabilities in cell

adhesion and receptor pharmacology.

AUTHOR: Edwards B.S.; Kuckuck F.W.; Prossnitz E.R.; Okun A.; Ransom

J.T.; Sklar L.A.
CORPORATE SOURCE: Dr. B.S. Edwards, Cytometry, UNM Health Sciences Center, 2325 Camino de Salud, Albuquerque, NM 87131, United States.
Bedwards@salud.unm.edu
SOURCE: Communications in Clinical Cytometry, (1 Mar 2001) 43/3 (211-216).
Refs: 15
ISSN: 0196-4763 CODEN: CCCYEM
COUNTRY: United States
DOCUMENT TYPE: Journal; Conference Article
FILE SEGMENT: 027 Biophysics, Bioengineering and Medical Instrumentation
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Background: Plug **flow cytometry** is a recently developed system for the automated delivery of multiple small boluses or "plugs" of cells or particles to the **flow cytometer** for analysis. Important system features are that **sample** plugs are of precisely defined volume and that the **sample** vessel need not be pressurized. We describe how these features enable direct cell concentration determinations and novel ways to integrate **flow cytometers** with other analytical instruments. Methods: Adhesion assays employed human polymorphonuclear neutrophils (PMNs) loaded with Fura Red and Chinese hamster ovary. . . were loaded with the fluorescent probe indo-1 for intracellular ionized calcium determinations.

A computer-controlled syringe or peristaltic pump loaded the **sample** into a **sample** loop of the plug **flow** coupler, a reciprocating eight-port valve. When the valve position was switched, the plug of **sample** in the **sample** loop was transported to the **flow cytometer** by a pressure-driven fluid line. Results: In stirred mixtures of PMNs and CHO cells, we used plug **flow cytometry** to directly quantify changes in concentrations of nonadherent singlet PMNs. This approach enabled accurate quantification of adherent PMNs in multicell aggregates. We constructed a novel plug **flow** interface between the **flow cytometer** and a cone-plate viscometer to enable real-time **flow cytometric** analysis of cell-cell adhesion under conditions of uniform shear. The **High Throughput** Pharmacology System (HTPS) is an instrument used for automated programming of complex pharmacological cell treatment protocols. It was interfaced via the plug **flow** coupling device to enable rapid (< 5 min) **flow cytometric** characterization of the intracellular calcium dose-response profile of U937 cells to formyl peptide. Conclusions: By facilitating the coupling of **flow cytometers** to other fluidics-based analytical instruments, plug **flow cytometry** has extended analytical capabilities in cell adhesion and pharmacological characterization of receptor-ligand interactions. .COPYRGT. 2001 Wiley-Liss, Inc.

L4 ANSWER 15 OF 63 USPATFULL
ACCESSION NUMBER: 2000:84104 USPATFULL
TITLE: Multiplexed molecular analysis apparatus and method
INVENTOR(S): Balch, William J., Woodlands, TX, United States
PATENT ASSIGNEE(S): Genometrix Inc., The Woodlands, TX, United States (U.S.
corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 6083763	20000704
APPLICATION INFO.:	US 1997-2170	19971231 (9)

	NUMBER	DATE
PRIORITY INFORMATION:	US 1996-34627	19961231 (60)

DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Chin, Christopher L.
LEGAL REPRESENTATIVE: Fish & Richardson P.C.
NUMBER OF CLAIMS: 27
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 27 Drawing Figure(s); 24 Drawing Page(s)
LINE COUNT: 2365
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method and apparatus for analyzing molecular structures within a **sample** substance using an array having a plurality of test sites upon which the **sample** substance is applied. The invention is also directed to a method and apparatus for constructing molecular arrays having a plurality of test sites. The invention allows for definitive high throughput analysis of multiple analytes in complex mixtures of **sample** substances. A combinatorial analysis process is described that results in the creation of an array of integrated chemical devices. These. . . a defined experiment. This approach is uniquely capable of rapidly providing a high density of information from limited amounts of **sample** in a cost-effective manner.

SUMM . . . a multiplexed molecular analysis apparatus and method for the detection and quantification of one or more molecular structures in a **sample**.

SUMM It is very desirable to rapidly detect and quantify one or more molecular structures in a **sample**. The molecular structures typically comprise ligands, such as antibodies and anti-antibodies. Ligands are molecules which are recognized by a particular. . .

SUMM . . . for a wide array of pathogens. In all of these cases there are fundamental constraints to the analysis, e.g., limited **sample**, time, or often both.

SUMM Multiplexing requires additional controls to maintain accuracy. False positive or negative results due to contamination, degradation of **sample**, presence of inhibitors or cross reactants, and inter/intra strand interactions should be considered when designing the analysis conditions.

SUMM . . . for completion. Of more concern is the potential for ambiguity when multiple strains of a pathogen are present in one **sample**. Virulence of the pathogen is often determined by the strain. An example is HPV, also known as human papilloma virus.. . .

SUMM . . . Southern and Northern analyses, have been used extensively as the primary method of detection for clinically relevant nucleic acids. The **samples** are prepared quickly to protect them from endogenous nucleases and then subjected to a restriction enzyme digest

or polymerase chain. . .

SUMM . . . clement is immobilized per well. This, of course, limits the amount of information that can be determined per unit of **sample** . Practical considerations, such as **sample** size, labor costs, and analysis time, place limits on the use of microplates in multiplex analyses. With only a single. . . format microplate. In the case where strain determination is to be made, multiple plates must be used. Distributing a patient **sample** over such a large number of wells becomes highly impractical due to limitations on available **sample** material. Thus, available patient **sample** volumes inherently limit the analysis and dilution of the **sample** to increase volume seriously affects sensitivity.

SUMM . . . the sensitivity of the assay, there are practical limitations to the number of sequences that can be amplified in a **sample**. For example, most multiplexed PCR reactions for clinical use do not amplify more than a few target sequences per reaction.. . . still be analyzed either by Sanger sequencing, gel electrophoresis, or hybridization techniques such as Southern blotting or microplate assays.

The **sample** components, by PCR's selective amplification, will be less likely to have aberrant results due to cross reactants. This will not. . . amplification positive control to ensure against false negatives. Inhibitors to the PCR process such as hemoglobin are common in clinical **samples**. As a result, the PCR process for multiplexed analysis is subject to most of the problems outlined previously. A high. . .

SUMM . . . array formats on solid surfaces, also called "chip formats." A large number of hybridization reactions using very small amounts of **sample** can be conducted using these chip formats thereby facilitating information rich analyses utilizing reasonable **sample** volumes.

SUMM 2) Non-amplification of target molecule due to the presence of small molecule inhibitors, degradation of **sample**, and/or high ionic strength.

SUMM . . . on the characteristics of the array of bound capture probe molecules, their complementary target molecules, and the nature of the **sample** matrix.

SUMM The multiplexed molecular analysis system of the instant invention is useful for analyzing and quantifying several molecular targets within a **sample** substance using an array having a plurality of biosites upon which the **sample** substance is applied. For example, this invention can be used with microarrays in a microplate for multiplexed diagnostics, drug discovery. . .

SUMM One application of the microplate based arrays of this invention is in parallel processing of a large number of **samples**. Large clinical labs process thousands of **samples** a day. A microplate configured with a four by four (4.times.4) matrix of biosites in each of the 96 wells would be able to perform a total of 1536 nearly simultaneous tests is from 96 different patient **samples** utilizing the proximal CCD imager as illustrated in FIG. 1. FIG. 1 is a diagram showing a multiplexed molecular analysis. . .

SUMM . . . without significant cross association to other macromolecules expected from either the patient or other organisms commonly associated with a specific **sample** type. Controls must be designed to prevent false positive or negative results from the sources outlined in the Background section.. . . For nucleic acid applications, these conditions are highly dependent on the capture probe length and composition, target base composition, and **sample** matrix. The number of arrays to be used depends on a number of different factors, e.g., the controls to be. . .

SUMM Another use of the hierarchical arrays and the reaction vessel based arrays would be for screening, **samples** for a broad range of possible targets. In one case, a diagnostic test is performed to search for the cause. . . probes for highly conserved nucleic acid regions. Results from this would indicate which additional array sets within the

microplate to **sample** next, moving to greater and greater specificity. If enough **sample** is available, as might be the case with donated blood or tissue, all of the decision tree elements could be interrogated simultaneously. If **sample** quantity is limiting, the approach could be directed in a serial fashion.

SUMM . . . specific to a particular analysis and consist of the appropriate array sets and the necessary fluidics to take a single **sample** and deliver the appropriate aliquot to each array in the set. The fluidics will deliver the appropriate association and wash. .

SUMM . . . designed in a standard 96 well microtiter plate format for room temperature operation to accommodate conventional robotic systems utilized for **sample** delivery and preparation. Also, the proximal CCD-based imager with a graphical user interface will enable the automation of the parallel. . .

DETD . . . detection" refers to the use of CCD technology for detection and imaging in which the CCD is proximal to the **sample** to be analyzed, thereby avoiding the need for conventional lenses.

DETD . . . number of diagnostic assays. Thus a set of parallel reactions can be handled with the same effort as a single **sample** in previously described methods. Hence, a greater number of assays can be handled within a fixed period of time. The. . .

DETD A. Preparing the **sample** for subsequent association to a probe array within the reaction chamber. This includes all front-end processes such as purification, isolation, denaturation and labeling required to extract the target molecules from the **sample**.

DETD . . . to determine information about the target molecules such as the presence and amount of specific molecular constituents within a given **sample** that leads to the analysis output.

DETD In this invention, the storage vessel allows for **sampling** either from a standard format microtiter plate or a customized plate designed to hold small volumes of liquid, allowing the. . .

DETD . . . for 1997) do not meet the needs for the drug discovery market, where a single company may screen over 100,000 **samples** per year.

DETD . . . and their spacing, with an emphasis on reducing the overall size as much as possible to reduce reagent costs and **sample** requirements. If a parallel printing approach is implemented using multiple pins or depositiors, the geometries of these depositors must allow. . .

DETD A **sample** protocol for the two piece approach is as follows:

DETD . . . on thin-film transistor arrays, amorphous silicon sensors, photodiode arrays, or the like. The array is disposed in proximity to the **sample** (target molecules hybridized to the biosites) and is comparable in size to the reaction chambers. In this manner, a relatively. . . spatial distribution of the bound target molecules is produced without requiring the use of one or more lenses between the **sample** and the imaging array. This apparatus offers:

DETD Moreover by placing the imaging array in proximity to the **sample** as illustrated in FIG. 1, the collection efficiency is improved by a factor of at least ten (100 over any lens-based technique such as found in conventional CCD cameras). Thus, the **sample** (emitter or absorber) is in near contact with the detector (imaging array), thereby eliminating conventional imaging optics such as lenses. . .

DETD . . . a static platform, whereby a plurality of imaging devices are arranged in a relatively large format area comparable to the **sample** size.

DETD . . . second subclass entails a dynamic platform that enables a smaller set of imaging devices to image a relatively large format **sample** by moving either the array of imaging devices or **sample**, relative to one another.

DETD . . . high resolution quantitative digital imaging and spectroscopy

of the spatial and/or temporal distribution of particle emissions or absorption from/by a **sample** (target molecules) in a relatively large format. The apparatus of this invention includes:

DETD b) a scanner for moving either the sensor array or the **sample** in a manner for efficient imaging; and

DETD c) a source of energy for exciting the **sample** or providing absorption by the **sample**.

DETD Optimally, the ratio of detector array size to **sample** image is one (1) for a static format and less than one (1) for a dynamic format.

DETD . . . responsible for obtaining the digital image from the sensor array and includes preamplification, amplification, analog to digital conversion, filtering, multiplexing, **sampling** and holding, and frame grabbing functions. Finally, the data processor processes the quantitative imaging data to provide the required parameters. . .

DETD . . . as illustrated in FIGS. 10A-10C. FIG. 10A depicts a CCD array with multiple pixels being exposed to a labeled biological **sample** 32 which causes the collection of electrons 34 beneath the respective pixel gate 16. Individual CCD arrays are closely aligned.

. .

DETD . . . the metal electrode 16. The filter is adapted to block the excitation radiation and pass the secondary emission from the **sample** 20. In a static platform embodiment, the sensor module remains fixed with respect to the **sample**. Hence to achieve the relatively large imaging format, a plurality of imaging devices CCD1 . .

. CCDN should be. . .

DETD As illustrated in FIG. 10A, a reaction vessel 20 is placed in proximity to the CCD array sensor 10. The **sample** can be excited by an external energy source or can be internally labeled with radioisotopes emitting energetic particles or radiation, or photons may be emitted by the **sample** when labeled with fluorescent and chemiluminescent substances. Conversely, direct absorption may be used to determine their presence. In this case, the absence of illuminating radiation on the detector may constitute the presence of a particular molecule structure.

Preferably the **sample** can be physically separated from the CCD detector by the faceplate which is transparent to the particle emission.

DETD . . . when the charged particles or radiation of energy hv shown by the asterisk 32 arising from or transmitted by the **sample** are incident (arrows 30) on the CCD gates 16. Alternatively, the CCDs can be constructed in a back illumination format. . .

DETD . . . isotopes (.sup.32 P, .sup.125 I). Consequently, the CCD is both a visible imager (applicable to fluorescent and chemiluminescent labeled molecular **samples**) and a particle spectrometer (applicable to radioisotope labeled **samples** as well as external x-ray radiated **samples**). Thus, the CCD can provide simultaneous imaging and spectroscopy in the same image.

DETD . . . Also, the scanning can be accomplished with intentional overlapping to provide continuous high resolution imaging across the entire large format **sample** area.

DETD . . . diagnostics. For immunoassays, the throughput of conventional ELISA assays can be increased with the multiplexed microplate format wherein a patient **sample** can be simultaneously interrogated by numerous antigens/antibodies within a single reaction chamber (well).

DETD Similarly for probe-based diagnostics, target molecules derived from a patient **sample** can be dispensed into a single well containing numerous biosites for diagnosing genetic or infectious diseases. For example, single-stranded nucleic. . . known mutations of cystic fibrosis are arranged within a single well in a microplate. Upon hybridization with the patient's DNA **sample**, the resulting

binding pattern obtained from the proximal CCD detector/imager indicates
the presence of such known mutations.

DETD . . . a number of sexually transmittable diseases within a single well (reaction chamber). Consequently for a single microtiter plate, numerous patient **samples** can be simultaneously interrogated each against a panel of numerous probes to provide a very rapid, cost effective diagnostic testing. . .

DETD . . . This format has other diagnostic advantages such as homogeneous
detection of amplified products without having to open or expose the **sample** well to the ambient environment.

DETD . . . In practice, 96 separate PCR amplification reactions would be carried out using genomic DNA templates isolated from 96 different patient **samples**. The figure illustrates the concept of genotyping starting with 96 previously robotically purified PCR templates from these reactions. Each purified. . .

DETD . . . jump over this specialized juncture, leaving the universal sequence as a single stranded motif. If a particular template in a **sample** well being amplified contains both primer loci (i.e., detection and capture sites), then a PCR product will be generated that.
. .

DETD . . . sequence attached to this primer serves as a sequence specific single stranded handle. When the template is present in the **sample** then sequence directed ligation will join both the label and the universal handle into a single product. After many cycles. .
.

DETD . . . digoxigenin, 2,4 dinitrophenol, and TRITC. Bispecific molecules
uniquely specific for both the immobilized hapten and another labeled analyte in the **sample** are added to each well. In this fashion, different multiple analytes can be simultaneously detected and their presence indicated by signals at specific hapten biosites. In this example, 96 individual **samples** can be assayed for four different analytes simultaneously. As shown, the fluorescein biosite detects a labeled receptor (protein) analyte, both. . . dinitrophenol
and digoxigenin haptens allow for the simultaneous detection or presence
of two additional types of protein receptors in the **sample**. Finally, the TRITC hapten allows for detection and presence of a specific enzyme substrate via an intervening enzyme conjugate. Once. .
.

DETD . . . molecular analysis system is also useful for analyzing the expression of hundreds of different mRNA species in a single tissue **sample** within a single well of a microtiter plate. Here synthetic nucleic acids form the distinct biosites which constitute numerous highly sensitive and selective hybridization analyses per **sample**, employing only 50 .mu.L of **sample** extract.
Such massive hybridization analyses enables the discovery and employment
of numerous biomarkers for specific diseases such as cancer. Essentially,. . . biomarkers. Once an mRNA biomarker set is discovered by this iterative approach, the technology is naturally suited for low cost, **high throughput** screening of large patient populations with the mRNA biomarker set of choice.

DETD . . . intact cells are analyzed utilizing the multiplexed format of this invention. Specifically, most "cell enrichment" protocols involve either double label **flow cytometry**, or physical separation of cells via affinity chromatography of some kind. Both require access to an antibody which is specific. . .

DETD The procedure is to add a complex cellular mixture, e.g., a biological **sample** (for example, blood or sputum), to such an antibody matrix, then with some local mixing, allowing the cells to bind. . . is nearly identical to the methods which are currently used to analyze

DNA or RNA in cells for microscopy or **flow cytometry**
.

DETD Basically, the procedure is initiated by preparing the microbial rRNA **sample** for hybridization to the biosite array within the reaction chamber. Following specific binding of the fluorescently labeled microbial RNA to the probe array, a two dimensional image results that uniquely characterizes the **sample**. The analyzer output is the microbial spectrum, consisting of the amount and type of microorganisms present in the **sample**.

DETD . . . Moreover, the proposed highly sensitive proximal CCD detection procedure, combined with the inherent amplification property of rRNA, reduces the combined **sample** preparation, assay, and detection time from days to hours.

DETD . . . high density arrays that support hundreds of immobilized probes per cm.sup.2 to facilitate multiple microorganism detection and identification in a **high throughput** manner.

DETD . . . and training is required since no cell culturing or gel-based sequencing is required. Instead, an operator merely subjects the prepared **sample** to automated hybridization, washing, and drying processes to obtain the microbial spectrum.

L4 ANSWER 24 OF 63 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 2000204238 MEDLINE
DOCUMENT NUMBER: 20204238
TITLE: **Flow cytometric** analysis of immunoprecipitates: **high-throughput** analysis of protein phosphorylation and protein-protein interactions.
AUTHOR: Lund-Johansen F; Davis K; Bishop J; de Waal Malefyt R
CORPORATE SOURCE: DNAX Research Institute for Cellular and Molecular Biology, Palo Alto, California, USA.. f-johans@online.no
SOURCE: CYTOMETRY, (2000 Apr 1) 39 (4) 250-9. Journal code: D92. ISSN: 0196-4763.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200008
ENTRY WEEK: 20000801
AB BACKGROUND: Activation-induced protein phosphorylation can be studied by Western blotting, but this method is time consuming and depends on the use of radioactive probes for quantitation. We present a novel assay for the assessment of protein phosphorylation based on latex particles and **flow cytometry**. METHODS: This method employs monoclonal antibodies coupled to latex particles to immobilize protein kinase substrates. Their phosphorylation status is assessed by reactivity with phosphoepitope-specific antibodies. The amount of immobilized protein on the particles was analyzed by direct or indirect immunofluorescence with antibodies to nonphosphorylated epitopes. RESULTS: The assay allowed measurement of phosphorylation of multiple protein kinase substrates in stimulated T cells, including the zeta chain of the T-cell receptor, ZAP-70, CD3, CD5, SHP-1, and ERK-2, using 1-3 microg of total cell protein per **sample**. The assay provided high resolution of kinetics of phosphorylation and dephosphorylation. Interactions of protein kinase substrates with associated signaling molecules were demonstrated. CONCLUSIONS: The novel assay allows **high-throughput** quantitative measurement of protein modifications during signal transduction. Copyright 2000 Wiley-Liss, Inc.

L4 ANSWER 25 OF 63 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 2000396053 MEDLINE
DOCUMENT NUMBER: 20318615
TITLE: **Flow cytometry**-based minisequencing: a new platform for **high-throughput** single-nucleotide polymorphism scoring.
AUTHOR: Cai H; White P S; Torney D; Deshpande A; Wang Z; Marrone B; Nolan J P
CORPORATE SOURCE: Bioscience Division, Los Alamos National Laboratory, Los Alamos, New Mexico, 87545, USA.
CONTRACT NUMBER: RR14101 (NCRR)
SOURCE: GENOMICS, (2000 Jun 1) 66 (2) 135-43. Journal code: GEN. ISSN: 0888-7543.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals

ENTRY MONTH: 200010
ENTRY WEEK: 20001003
AB Single-nucleotide polymorphisms (SNPs) are the most abundant type of human genetic variation. These variable sites are present at high density in the genome, making them powerful tools for mapping and diagnosing disease-related alleles. We have developed a sensitive and rapid flow cytometry-based assay for the multiplexed analysis of SNPs based on polymerase-mediated primer extension, or minisequencing, using microspheres as solid supports. The new method involves subnanomolar concentrations of **sample** in small volumes (approximately 10 &mgr;l) which can be analyzed at rates of one **sample** per minute or faster, without a wash step. Further, genomic analysis using multiplexing microsphere arrays (GAMMArrays), enables the simultaneous analysis of dozens, and potentially hundreds of SNPs per **sample**. We have tested the new method by genotyping the Glu69 variant from the HLA DPB1 locus, a SNP associated with chronic beryllium disease, as well as HLA DPA1 alleles using the multiplexed method. The results demonstrate the sensitivity and accuracy of flow cytometry-based minisequencing, a powerful new tool for genome- and global-scale SNP analysis.

L4 ANSWER 26 OF 63 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 2000145745 MEDLINE
DOCUMENT NUMBER: 20145745
TITLE: Multiplexed single nucleotide polymorphism genotyping by oligonucleotide ligation and flow cytometry.
AUTHOR: Iannone M A; Taylor J D; Chen J; Li M S; Rivers P; Slentz-Kesler K A; Weiner M P
CORPORATE SOURCE: Department of Molecular Sciences, Glaxo Wellcome Research Laboratories, Research Triangle Park, NC 27709-3398, USA.. mai49583@glaxowellcome.com
SOURCE: CYTOMETRY, (2000 Feb 1) 39 (2) 131-40.
Journal code: D92. ISSN: 0196-4763.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200005
ENTRY WEEK: 20000502
AB BACKGROUND: We have developed a rapid, **high throughput** method for single nucleotide polymorphism (SNP) genotyping that employs an oligonucleotide ligation assay (OLA) and **flow cytometric** analysis of fluorescent microspheres. METHODS: A fluoresceinated oligonucleotide reporter sequence is added to a "capture" probe by OLA. Capture probes are designed to hybridize both to genomic "targets" amplified by polymerase chain reaction and to a separate complementary DNA sequence that has been coupled to a microsphere. These sequences on the capture probes are called "ZipCodes". The OLA-modified capture probes are hybridized to ZipCode complement-coupled microspheres. The use of microspheres with different ratios of red and orange fluorescence makes a multiplexed format possible where many SNPs may be analyzed in a single tube. **Flow cytometric** analysis of the microspheres simultaneously identifies both the microsphere type and the fluorescent green signal associated with the SNP genotype. RESULTS: Application of this methodology is demonstrated by the multiplexed genotyping of seven CEPH DNA **samples** for nine SNP markers located near the ApoE locus on chromosome 19. The microsphere-based SNP analysis agreed with genotyping by sequencing in all cases. CONCLUSIONS: Multiplexed SNP genotyping by OLA with **flow cytometric** analysis of fluorescent microspheres is an accurate and rapid method for the analysis of SNPs.

L4 ANSWER 29 OF 63 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 2000175699 MEDLINE
DOCUMENT NUMBER: 20175699
TITLE: Enumeration of micronucleated reticulocytes in rat peripheral blood: a flow cytometric study.
AUTHOR: Torous D K; Dertinger S D; Hall N E; Tometsko C R
CORPORATE SOURCE: Litron Laboratories, 1351 Mount Hope Avenue, Rochester, NY 14620, USA.
CONTRACT NUMBER: R43 ES07707-02 (NIEHS)
SOURCE: MUTATION RESEARCH, (2000 Feb 16) 465 (1-2) 91-9.
Journal code: NNA. ISSN: 0027-5107.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 200006
ENTRY WEEK: 20000603
AB Micronuclei (MN) are routinely enumerated in mouse peripheral blood to index genotoxicity. Recent data from the Collaborative Study Group for the Micronucleus Test (CSGMT) [CSGMT (The Collaborative Study Group for the Micronucleus Test), Evaluation of the rat micronucleus test with bone marrow and peripheral blood: summary of the 9th collaborative study by CSGMT/JEMS MMS, Environ. Mol. Mutagen. 32 (1998) 84-100] suggest that rat peripheral blood may also be appropriate for the enumeration of MN, if scoring is limited to the youngest fraction of reticulocytes. The experiments described herein were designed to test whether modifications to a **flow cytometric** scoring procedure for measuring micronucleated reticulocytes (MN-RET) in mouse peripheral blood could be extended to accurately enumerate MN in rat peripheral blood. Rats were treated with saline or one of three genotoxic agents (6-mercaptopurine, ethyl methanesulfonate or propane sultone) in an acute dosing protocol. Peripheral blood **samples** were subsequently collected for both microscopic and **flow cytometric** analysis. Micronucleus frequencies were scored in the youngest fraction of reticulocytes: scoring by microscopy was restricted to the types I and II reticulocytes based on RNA content utilizing acridine orange supravital staining; **flow cytometric** measurements were restricted to the youngest fraction of reticulocytes based on transferrin receptor (CD71) staining. A statistically significant dose-related increase in the incidence of MN was observed, irrespective of scoring method. A higher level of statistical discrimination between control and genotoxin-treated groups was observed for the **flow cytometric** data and can most likely be explained by the increased number of cells scored (10x more than microscopy) and the lower scoring variability. Together, these data suggest that (i) rat peripheral blood represents an appropriate compartment for evaluating genotoxin-induced MN when the analysis is restricted to young reticulocytes, and (ii) the measurement of MN in rat peripheral blood reticulocytes benefits from the **high throughput** methodology of **flow cytometry**.

L4 ANSWER 30 OF 63 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 2000121085 MEDLINE
DOCUMENT NUMBER: 20121085
TITLE: **High-throughput flow cytometric** DNA fragment sizing.
AUTHOR: Van Orden A; Keller R A; Ambrose W P
CORPORATE SOURCE: Bioscience Division, Los Alamos National Laboratory, New Mexico 87545, USA.
SOURCE: ANALYTICAL CHEMISTRY, (2000 Jan 1) 72 (1) 37-41.
Journal code: 4NR. ISSN: 0003-2700.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English
ENTRY MONTH: 200006
ENTRY WEEK: 20000602

AB The rate of detection and sizing of individual fluorescently labeled DNA fragments in conventional single-molecule flow cytometry (SMFC) is limited by optical saturation, photon-counting statistics, and fragment overlap to approximately 100 fragments/s. We have increased the detection rate for DNA fragment sizing in SMFC to approximately 2000 fragments/s by parallel imaging of the fluorescence from individual DNA molecules, stained with a fluorescent intercalating dye, as they passed through a planar sheet of excitation laser light, resulting in order of magnitude improvements in the measurement speed and the **sample** throughput compared to conventional SMFC. Fluorescence bursts were measured from a fM solution of DNA fragments ranging in size from 7 to 154 kilobase pairs. A data acquisition time of only a few seconds was sufficient to determine the DNA fragment size distribution. A linear relationship between the number of detected photons per burst and the DNA fragment size was confirmed. Application of this parallel fluorescence imaging method will lead to improvements in the speed, throughput, and sensitivity of other types of flow-based analyses involving the study of single molecules, chromosomes, cells, etc.

L4 ANSWER 31 OF 63 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 9
ACCESSION NUMBER: 2000344416 EMBASE
TITLE: Commercial high speed machines open new opportunities in **high throughput flow cytometry** (HTFC).
AUTHOR: Ashcroft R.G.; Lopez P.A.
CORPORATE SOURCE: R.G. Ashcroft, P.O. Box 207, Black Rock, Vic. 3193, United States. cytomat@netcore.com.au
SOURCE: Journal of Immunological Methods, (21 Sep 2000) 243/1-2 (13-24).
Refs: 40
ISSN: 0022-1759 CODEN: JIMMBG
PUBLISHER IDENT.: S 0022-1759(00)00219-2
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 026 Immunology, Serology and Transplantation
027 Biophysics, Bioengineering and Medical Instrumentation
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Two recent events have opened a new domain of **flow cytometry** applications which we term **high throughput flow cytometry** (HTFC). The release of a commercial high speed sorter in 1994 placed HTFC within the reach of anyone who could buy one of the new machines and not just the handful of advanced laboratories worldwide that had custom built their own high speed sorters. The advent in 1999 of HTFC analysis capabilities of 100 000 cells/s marks the second stage in this enabling of HTFC. We describe the technical basis of HTFC. The commercial high speed sorters measure cells in dead-times three to six times shorter than conventional machines. They can sort with high yield and high purity at rates from 25 000 to 60 000 cells/s, depending on their settings, mainly by virtue of their use of high drop creation rates 100 000 drops/s or more. Finally, one series can analyse the measured cells at rates exceeding these sort-rates and at least six times faster than conventional sorters could. The performance of the systems made by the three manufacturers can be readily assessed for single laser systems. Comparison becomes difficult for multiple beam machines, due to requirements for multi- beam **sampling** for each cell and due to the demands of fluorescence compensation between signals from one laser and between signals from two or three lasers. Applications are described in the field of rare cell analysis and isolation as well as from sorting of abundant cell populations. (C) 2000 Elsevier Science B.V.

L4 ANSWER 32 OF 63 USPATFULL
ACCESSION NUMBER: 1999:166808 USPATFULL
TITLE: Assay and kit for determining the condition of cells
INVENTOR(S): Crouch, Sharon Patricia Mary, The Park, United Kingdom
Slater, Kevin John, The Park, United Kingdom
Sowter, David Peter, Hucknall, United Kingdom
PATENT ASSIGNEE(S): BTG International Limited, London, United Kingdom (non-U.S. corporation)

	NUMBER	DATE	
PATENT INFORMATION:	US 6004767	19991221	
APPLICATION INFO.:	US 1999-326578	19990607	(9)

RELATED APPLN. INFO.: Continuation of Ser. No. WO 1997-GB3556, filed on 24 Dec 1997

	NUMBER	DATE
PRIORITY INFORMATION:	GB 1996-26932	19961224

DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Leary, Louise N.
LEGAL REPRESENTATIVE: Nixon & Vanderhye
NUMBER OF CLAIMS: 20
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 6 Drawing Figure(s); 6 Drawing Page(s)
LINE COUNT: 1065
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to a method of assay and a kit for carrying out the assay for determining the physical condition of biological cells in vitro. The assay method distinguishes whether cells are alive and proliferating or are dying and, if dying, whether the cells are in an apoptotic or necrotic condition.

L4 ANSWER 34 OF 63 USPATFULL
ACCESSION NUMBER: 1999:136974 USPATFULL
TITLE: Drug screening process
INVENTOR(S): Hochman, Daryl W., Seattle, WA, United States
PATENT ASSIGNEE(S): Cytoscan Sciences, L.L.C., Seattle, WA, United States (U.S. corporation)

	NUMBER	DATE	
PATENT INFORMATION:	US 5976825	19991102	
APPLICATION INFO.:	US 1997-949416	19971014	(8)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1995-539296, filed on 4 Oct 1995
DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Kunz, Gary L.
LEGAL REPRESENTATIVE: Speckman, Ann W.; Sleath, Janet
NUMBER OF CLAIMS: 12
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 3 Drawing Figure(s); 6 Drawing Page(s)
LINE COUNT: 1275
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB There is disclosed a method for screening drug candidate compounds for anti-epileptic activity, a method for screening drug candidate compounds for activity to prevent or treat symptoms of Alzheimer's disease, and a method for determining cell viability and health of living cells inside polymeric tissue implants.

L4 ANSWER 37 OF 63 USPATFULL
ACCESSION NUMBER: 1999:56404 USPATFULL
TITLE: Drug screening process measuring changes in cell volume
INVENTOR(S): Hochman, Daryl W., Seattle, WA, United States
PATENT ASSIGNEE(S): Cytoscan Sciences LLC, Mercer Island, WA, United States
(U.S. corporation)

	NUMBER	DATE	
PATENT INFORMATION:	US 5902732	19990511	
APPLICATION INFO.:	US 1995-539296	19951004	(8)

DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Kunz, Gary L.
LEGAL REPRESENTATIVE: Oster, Jeffrey B.
NUMBER OF CLAIMS: 14

EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 33 Drawing Figure(s); 6 Drawing Page(s)
LINE COUNT: 1272
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB There is disclosed a method for screening drug candidate compounds for anti-epileptic activity, a method for screening drug candidate compounds for activity to prevent or treat symptoms of Alzheimer's disease, and a method for determining cell viability and health of living cells inside polymeric tissue implants.

L4 ANSWER 39 OF 63 USPATFULL
ACCESSION NUMBER: 1999:3863 USPATFULL
TITLE: Apparatus and method for performing electrodynamic focusing on a microchip
INVENTOR(S): Ramsey, John Michael, Knoxville, TN, United States
Jacobson, Stephen C., Knoxville, TN, United States
PATENT ASSIGNEE(S): Lockheed Martin Energy Systems, Inc., Oak Ridge, TN, United States (U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 5858187	19990112
APPLICATION INFO.:	US 1996-721264	19960926 (8)

DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Beisner, William H.
ASSISTANT EXAMINER: Stavsiak, Jr., John S.
LEGAL REPRESENTATIVE: Dann Dorfman Herrell and SkillmanMorgan & Finnegan
NUMBER OF CLAIMS: 21
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 22 Drawing Figure(s); 11 Drawing Page(s)
LINE COUNT: 678
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A microchip device includes a focusing channel, in which an electric field strength established in the focusing channel is controlled relative to an electric field strength established in a material transport channel segment to spatially focus the material traversing the material transport channel segment.

L4 ANSWER 42 OF 63 MEDLINE DUPLICATE 12
ACCESSION NUMBER: 1999418873 MEDLINE
DOCUMENT NUMBER: 99418873
TITLE: Plug flow cytometry: An automated coupling device for rapid
sequential flow cytometric **sample** analysis.
AUTHOR: Edwards B S; Kuckuck F; Sklar L A
CORPORATE SOURCE: Cancer Research and Treatment Center, Departments of Cytometry and Pathology, University of New Mexico Health Sciences Center, Albuquerque, New Mexico 87131, USA.
CONTRACT NUMBER: RR11830 (NCRR)
HL56384 (NHLBI)
RR01315 (NCRR)
SOURCE: CYTOMETRY, (1999 Oct 1) 37 (2) 156-9.
Journal code: D92. ISSN: 0196-4763.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200001
ENTRY WEEK: 20000104
AB BACKGROUND: The tools for **high throughput flow cytometry** have been limited in part because of the requirement that the **samples** must **flow** under pressure. We describe a simple system for **sampling** repetitively from an open vessel. METHODS: Under computer control, the **sample** is loaded into a **sample** loop in a reciprocating eight-way valve by the action of a syringe. When the valve position is switched, the plug of **sample** in the **sample** loop is transported to the **flow cytometer** by a pressure-driven fluid line. By coupling the plug-forming capability to a second multi-port valve, **samples** can be delivered sequentially from separate vessels. RESULTS: The valve is able to deliver **samples** at rates ranging up to about 9 **samples** per minute. Each plug of **sample** has uniform delivery characteristics with a reproducible coefficient of variation (CV). Even at the highest **sampling** rate, carryover between **samples** is limited. CONCLUSIONS: Plug-**flow flow cytometry** has the potential to automate the delivery of small **samples** from unpressurized sources at rates compatible with many screening and assay applications. Copyright 1999 Wiley-Liss, Inc.

L4 ANSWER 43 OF 63 MEDLINE DUPLICATE 13
ACCESSION NUMBER: 2000019611 MEDLINE
DOCUMENT NUMBER: 20019611
TITLE: Sheath fluid control to permit stable flow in rapid mix flow cytometry.
AUTHOR: Seamer L C; Kuckuck F; Sklar L A
CORPORATE SOURCE: University of New Mexico, Cancer Research and Treatment Center, Albuquerque 87131, USA.
CONTRACT NUMBER: RR01315 (NCRR)
SOURCE: CYTOMETRY, (1999 Jan 1) 35 (1) 75-9.
Journal code: D92. ISSN: 0196-4763.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200001
ENTRY WEEK: 20000104

AB BACKGROUND: **Flow cytometry** is a potentially powerful tool to analyze the kinetics of ligand binding, cell response and molecular assembly. The difficulty in adding reactant to cells, achieving adequate mixing, delivering those cells to the laser focal point and establishing stable **flow**, has historically limited **flow cytometry** to systems with reactions times longer than 5 s. With the advent of automated syringes and **flow** injection methods, **sample** injection times shorter than 1 s have become routine. However, an inherent problem in acquiring time courses starting under 1 s is that rapid **sample** introduction through the **flow** tip to the detection point perturbs laminar **flow**. The purpose of this work was to determine if stable **flow** could be reestablished more quickly if the sheath **flow** was reduced during **sample** introduction, returning to normal sheath and **sample** rates afterward. METHODS: We used programmable syringes and valves to control **sample** mixing as well as sheath and **sample** delivery through the **flow** tip to the detection point for stream-in-air detection. Stable **flow** was monitored by mean particle fluorescence during **sample** introduction. RESULTS: With no sheath reduction, stable **flow** recovered after more than 1 s. By reducing sheath **flow** during the short period (300 msec) of **sample** mixing and delivery, stable laminar **flow** recovered within 200 msec. CONCLUSIONS: This use of automated syringes to control both sheath and **sample flow** provides a potential for robust **sample** handling applicable to kinetic as well as **high throughput flow cytometric** analysis.

L4 ANSWER 44 OF 63 USPATFULL

ACCESSION NUMBER: 1998:122274 USPATFULL

TITLE: Automated method and device for identifying and quantifying platelets and for determining platelet activation state using whole blood **samples**

INVENTOR(S): Zelmanovic, David, Monsey, NY, United States
Colella, Gregory M., Montclair, NJ, United States
Hetherington, Edward J., Brewster, NY, United States
Chapman, Evelyn Sabrinah, Croton-on-Hudson, NY, United States
Paseltiner, Lynn, Monroe, NY, United States

PATENT ASSIGNEE(S): Bayer Corporation, Tarrytown, NY, United States (U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 5817519	19981006
APPLICATION INFO.:	US 1996-742889	19961101 (8)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1995-581293, filed on 28 Dec 1995, now abandoned

DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Pyon, Harold Y.
LEGAL REPRESENTATIVE: Morgan & Finnegan, L.L.P.
NUMBER OF CLAIMS: 73
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 33 Drawing Figure(s); 14 Drawing Page(s)
LINE COUNT: 2204

AB The present invention provides a highly sensitive and accurate method and system for the discrimination and quantification of platelets in a whole blood **sample** using automated hematology instruments. The method and system of the invention provide the accurate measurements of platelet dry mass and platelet component concentration in both normal blood **samples** and in abnormal blood **samples**, such as those from thrombocytopenic patients. The determination of platelet dry mass and platelet component concentration can serve to assess the activation state of platelets since activated platelets possess measurably lower component concentrations and refractive indices than do

unactivated platelets. The method and system of the invention also allows the clinician or skilled practitioner to determine the age of a blood **sample** on the basis of the measured parameter of platelet component concentration.

L4 ANSWER 45 OF 63 USPATFULL
ACCESSION NUMBER: 1998:98755 USPATFULL
TITLE: Methods for identifying compounds useful in treating type II diabetes
INVENTOR(S): Glucksmann, M. Alexandra, Somerville, MA, United States
PATENT ASSIGNEE(S): Millennium Pharmaceuticals, Inc., Cambridge, MA, United States (U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 5795726	19980818
APPLICATION INFO.:	US 1997-782047	19970110 (8)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1996-760246, filed on 4 Dec 1996 which is a continuation-in-part of Ser. No. US 1996-749431, filed on 15 Nov 1996 which is a continuation-in-part of Ser. No. US 1996-748229, filed on 12 Nov 1996, now abandoned
DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Saunders, David
ASSISTANT EXAMINER: VanderVegt, F. Pierre
LEGAL REPRESENTATIVE: Arnold, Esq., Beth E.Foley, Hoag & Eliot LLP
NUMBER OF CLAIMS: 10
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 5 Drawing Figure(s); 5 Drawing Page(s)
LINE COUNT: 4150
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Methods for identifying compounds, which modulate the bioactivity of human hepatic nuclear factor-1 (HNF-1), and which are therefore useful in treating type II diabetes are disclosed.

L4 ANSWER 47 OF 63 USPATFULL
ACCESSION NUMBER: 1998:78960 USPATFULL
TITLE: Simultaneous human ABO and RH(D) blood typing or antibody screening by flow cytometry
INVENTOR(S): Vyas, Girish N., San Francisco, CA, United States
Venkateswaran, Kodumudi, San Francisco, CA, United States
PATENT ASSIGNEE(S): The Regents of the University of California, Oakland, CA, United States (U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 5776711	19980707
APPLICATION INFO.:	US 1996-747558	19961112 (8)

DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Scheiner, Toni R.
LEGAL REPRESENTATIVE: Bozicevic & Reed LLP; Bozicevic, Esq., Karl
NUMBER OF CLAIMS: 18
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 12 Drawing Figure(s); 4 Drawing Page(s)
LINE COUNT: 729
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Flow cytometric methodology is provided for simultaneous determination of (1) ABO and Rh(D) typing of human red cells, (2) natural isoantibodies in plasma, and (3) screening for alloantibodies in plasma.
The method includes (a) the use of a unique combination of fluorescent labelled antibodies to A, B and Rh(D) antigens to carry out (1); (b) different sized beads coated with blood group substances A & B to carry

out (2); and (c) the differential fluorescent labelling of screening reagent red blood cells for flow cytometric analyses to carry out (3). The routine ABO and Rh(D) typing and antibody screening of human blood for both isoantibodies and alloantibodies can be determined in three individual reactions compared to 7 to 10 tests currently performed in blood banks.

L4 ANSWER 48 OF 63 USPATFULL

ACCESSION NUMBER:	1998:78958 USPATFULL
TITLE:	Method for preparation and analysis of leukocytes in whole blood
INVENTOR(S):	Jackson, Anne Louise, Ridgefield, WA, United States Hoffman, Robert Alan, Livermore, CA, United States Blidy, Andrew D., Redwood City, CA, United States Murchison, Kenneth Earl, Ben Lomond, CA, United States Bierre, Pierre, Redwood City, CA, United States Thiel, Dan E., Pleasanton, CA, United States
PATENT ASSIGNEE(S):	Becton Dickinson and Company, Franklin Lakes, NJ, United States (U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 5776709	19980707
APPLICATION INFO.:	US 1994-286094	19940804 (8)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1993-15759, filed on 10 Feb 1993, now abandoned Continuation-in-part of Ser. No. US 1992-846316, filed on 5 Mar 1992, now abandoned which is a continuation-in-part of Ser. No. US 1991-751020, filed on 28 Aug 1991, now abandoned

DOCUMENT TYPE:	Utility
PRIMARY EXAMINER:	Spiegel, Carol A.
LEGAL REPRESENTATIVE:	Capello, Susan A.; Ronning, Jr., Royal N.
NUMBER OF CLAIMS:	2
EXEMPLARY CLAIM:	1
NUMBER OF DRAWINGS:	20 Drawing Figure(s); 10 Drawing Page(s)
LINE COUNT:	965

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method of flow cytometric analysis of leukocyte subpopulations using a fluorescence trigger and gating on light scatter vs. fluorescence. The methods are useful where light scatter parameters are unsatisfactory for identification of leukocyte subpopulations, for example when analyzing lysed blood **samples** without removal of lysing reagent or unbound label prior to analysis.

L4 ANSWER 49 OF 63 USPATFULL

ACCESSION NUMBER:	1998:75447 USPATFULL
TITLE:	Methods for the rapid analysis of the reticulocytes
INVENTOR(S):	Kim, Young Ran, Sunnyvale, CA, United States Kantor, Johanna, Palo Alto, CA, United States
PATENT ASSIGNEE(S):	Abbott Laboratories, Abbott Park, IL, United States (U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 5773299	19980630
APPLICATION INFO.:	US 1996-777727	19961220 (8)

RELATED APPLN. INFO.: Division of Ser. No. US 1995-426408, filed on 21 Apr 1995

DOCUMENT TYPE:	Utility
PRIMARY EXAMINER:	McMahon, Timothy
ASSISTANT EXAMINER:	Carrillo, Sharidan
LEGAL REPRESENTATIVE:	Poulos, Nicholas A.; Casuto, Dianne

NUMBER OF CLAIMS: 11
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 25 Drawing Figure(s); 15 Drawing Page(s)
LINE COUNT: 1051
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method and reagent for the simultaneous or independent enumeration of reticulocytes in a whole blood **sample**, without the need to separately incubate the **sample** and reagent. The reagent contains a reticulocyte staining amount of an unsymmetrical cyanine dye, from about 40 mM to about 60 mM of a buffer selected from the group consisting of imidazole, Tris and Bis-Tris and a dye stabilizing amount of a non-ionic surfactant selected from the group consisting of N, N-bis[3-D-Glucon-amidopropyl] cholamide and a polyoxypropylene-polyoxyethylene block copolymer. The reagent has a pH from about 6.8 to about 7.2 and an osmolarity adjusted to about 280 to about 310 mosm/l with a mono-, or di-, valent alkali salt selected from the group consisting of NaCl, KCl, LiCl, CaCl.sub.2, MgCl.sub.2 and ZnCl.sub.2. The method utilizes the reagent in a no incubation process that also allows for the simultaneous determination of CBC as well as reticulocyte counts and maturity indices.

L4 ANSWER 51 OF 63 USPATFULL
ACCESSION NUMBER: 97:120509 USPATFULL
TITLE: Flow sorter with video-regulated droplet spacing
INVENTOR(S): Sweet, Richard G., Palo Alto, CA, United States
PATENT ASSIGNEE(S): Becton Dickinson and Company, Franklin Lakes, NJ, United States (U.S. corporation)

	NUMBER	DATE	
PATENT INFORMATION:	US 5700692	19971223	
APPLICATION INFO.:	US 1994-312592	19940927	(8)

DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Le, Long V.
LEGAL REPRESENTATIVE: Wark, Allen W.
NUMBER OF CLAIMS: 4
EXEMPLARY CLAIM: 3
NUMBER OF DRAWINGS: 3 Drawing Figure(s); 3 Drawing Page(s)
LINE COUNT: 626

AB A droplet-deflection flow sorter images a series of droplets, analyzes the image to determine droplet spacing, and alters flow velocity to achieve a desired spacing. Cells in a suspension are forced by gas pressure into a narrow conduit to serialize them and then out a nozzle that includes a "window" that allows cells of interest to be characterized. Droplets breaking off from the exiting jet are imaged by a video system including a strobed light source and a video camera. Droplet spacing is determined by locating the centers of gravity of the droplets. The centers of gravity are located by processing the droplet images to produce line segments corresponding to volumetric droplet slices, with the relative volumes of the slices being proportional to the squares of the line segment lengths. This approach determines droplet center positions and thus spacing more accurately than non-imaging methods, especially with aspherical droplets and droplets with satellites. The actual droplet spacing is compared to desired spacing, and the pressure regulator is adjusted accordingly. Synchronously, voltage is applied to droplets of interest. The droplets pass between a pair of deflection plates with a potential difference between them, so that charged droplets containing cells of interest are deflected and gathered while uncharged droplets not containing such cells are not deflected.

L4 ANSWER 52 OF 63 USPATFULL
ACCESSION NUMBER: 97:109767 USPATFULL
TITLE: Compositions and methods for the rapid analysis of reticulocytes
INVENTOR(S): Kim, Young Ran, Sunnyvale, CA, United States
Kantor, Johanna, Palo Alto, CA, United States
PATENT ASSIGNEE(S): Abbott Laboratories, Abbott Park, IL, United States (U.S. corporation)

	NUMBER	DATE	
PATENT INFORMATION:	US 5691204	19971125	
APPLICATION INFO.:	US 1995-426408	19950421	(8)

DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Warden, Jill
ASSISTANT EXAMINER: Carrillo, Sharidan
LEGAL REPRESENTATIVE: Poulos, Nicholas A.
NUMBER OF CLAIMS: 15
EXEMPLARY CLAIM: 13

NUMBER OF DRAWINGS: 25 Drawing Figure(s); 15 Drawing Page(s)
LINE COUNT: 1003
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A method and reagent for the simultaneous or independent enumeration of reticulocytes in a whole blood **sample**, without the need to separately incubate the **sample** and reagent. The reagent contains a reticulocyte staining amount of an unsymmetrical cyanine dye, from about 40 mM to about 60 mM of a buffer selected from the group consisting of imidazole, Tris and Bis-Tris and a dye stabilizing amount of a non-ionic surfactant selected from the group consisting of N, N-bis[3-D-Glucon-amidopropyl] cholamide and a polyoxypropylene-polyoxyethylene block copolymer. The reagent has a pH from about 6.8 to about 7.2 and an osmolarity adjusted to about 280 to about 310 mOsm/l with a mono-, or di-, valent alkali salt selected from the group consisting of NaCl, KCl, LiCl, CaCl.sub.2, MgCl.sub.2 and ZnCl.sub.2. The method utilizes the reagent in a no incubation process that also allows for the simultaneous determination of CBC as well as reticulocyte counts and maturity indices.

L4 ANSWER 53 OF 63 USPATFULL
ACCESSION NUMBER: 97:91404 USPATFULL
TITLE: Methods and apparatus for DNA sequencing
INVENTOR(S): Ulmer, Kevin M., Cohasset, MA, United States
PATENT ASSIGNEE(S): SEQ, Ltd., Princeton, NJ, United States (U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 5674743	19971007
APPLICATION INFO.:	US 1995-463831	19950605 (8)

RELATED APPLN. INFO.: Division of Ser. No. US 1995-376761, filed on 23 Jan 1995 which is a continuation of Ser. No. US 1993-12862, filed on 1 Feb 1993, now abandoned
DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Redding, David A.
LEGAL REPRESENTATIVE: Pennie & Edmonds
NUMBER OF CLAIMS: 27
EXEMPLARY CLAIM: 25
NUMBER OF DRAWINGS: 15 Drawing Figure(s); 13 Drawing Page(s)
LINE COUNT: 3716
AB The present invention provides a method and apparatus for automated DNA sequencing. The method of the invention includes the steps of: a) using a processive exonuclease to cleave from a single DNA strand the next available single nucleotide on the strand; b) transporting the single nucleotide away from the DNA strand; c) incorporating the single nucleotide in a fluorescence-enhancing matrix; d) irradiating the single nucleotide to cause it to fluoresce; e) detecting the fluorescence; f) identifying the single nucleotide by its fluorescence; and g) repeating steps a) to f) indefinitely (e.g., until the DNA strand is fully cleaved or until a desired length of the DNA is sequenced). The apparatus of the invention includes a cleaving station for the extraction of DNA from cells and the separation of single nucleotides from the DNA; a transport system to separate the single nucleotide from the DNA and incorporate the single nucleotide in a fluorescence-enhancing matrix; and a detection station for the irradiation, detection and identification of the single nucleotides. The nucleotides are advantageously detected by irradiating the nucleotides with a laser to stimulate their natural fluorescence, detecting the fluorescence spectrum and matching the detected spectrum with that previously recorded for the four nucleotides

in order to identify the specific nucleotide.

L4 ANSWER 54 OF 63 USPATFULL
ACCESSION NUMBER: 97:51857 USPATFULL
TITLE: Synthesizing and screening molecular diversity
INVENTOR(S): Dower, William J., Menlo Park, CA, United States
Barrett, Ronald W., Sunnyvale, CA, United States
Gallop, Mark A., Palo Alto, CA, United States
Needels, Michael C., Oakland, CA, United States
PATENT ASSIGNEE(S): Affymax Technologies N.V., Curacao, Netherlands Antilles (non-U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 5639603	19970617
APPLICATION INFO.:	US 1993-146886	19931102 (8)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1992-946239, filed on 16 Sep 1992 which is a continuation-in-part of Ser. No. US 1991-762522, filed on 18 Sep 1991, now abandoned
DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Fleisher, Mindy
ASSISTANT EXAMINER: Ketter, James
LEGAL REPRESENTATIVE: Kaster, Kevin; Norviel, Vern; Stevens, Lauren L.
NUMBER OF CLAIMS: 14
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 1 Drawing Figure(s); 1 Drawing Page(s)
LINE COUNT: 3125
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A general stochastic method for synthesizing compounds can be used to generate large collections of tagged compounds that can be screened to identify and isolate compounds with useful properties.

L4 ANSWER 56 OF 63 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 15
ACCESSION NUMBER: 97025957 EMBASE
DOCUMENT NUMBER: 1997025957
TITLE: Simple and reliable enumeration of micronucleated reticulocytes with a single-laser flow cytometer.
AUTHOR: Dertinger S.D.; Torous D.K.; Tometsko K.R.
CORPORATE SOURCE: S.D. Dertinger, Litron Laboratories, 1351 Mount Hope Avenue, Rochester, NY 14620, United States
SOURCE: Mutation Research - Genetic Toxicology, (1996) 371/3-4 (283-292).
Refs: 27
ISSN: 0165-1218 CODEN: MGTOEB
PUBLISHER IDENT.: S 0165-1218(96)00130-9
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 022 Human Genetics
052 Toxicology
LANGUAGE: English
SUMMARY LANGUAGE: English
AB A **flow cytometric** procedure for scoring micronuclei in mouse peripheral blood erythrocytes, especially reticulocytes, is described. The methods reported herein were developed in an effort to simplify the techniques and to reduce the equipment requirements associated with automated micronucleus analyses. With this procedure, fluorescein-conjugated monoclonal antibodies which bind to the CD71-defined antigen (the transferrin receptor) are used to label reticulocytes. The nucleic acid dye propidium iodide is used to identify cells with micronuclei. Given 488 nm excitation, four populations of erythrocytes are clearly resolved: normochromatic erythrocytes with and without micronuclei, and reticulocytes with and without micronuclei. Since the method is capable of simultaneously providing the incidence of micronuclei in both mature and immature erythrocyte populations, it is

compatible with either chronic or acute treatment regimens. To demonstrate cell handling and **flow cytometric** procedures for quantitatively analyzing peripheral blood micronuclei, an experiment with the model clastogen methyl methanesulfonate is described. Additionally, a reconstruction experiment was performed whereby three mouse blood **samples** were spiked with successively greater volumes of blood from a clastogen-treated animal so each preparation differed slightly, but definitely, in micronucleus content. Each **sample** was scored six times by conventional microscopy and by **flow cytometry** so that the two methods could be directly compared. Collectively, the results from the methyl methanesulfonate experiment and the reconstruction study demonstrate the accuracy and reliability of the **flow cytometric** method. Furthermore, advantages associated with objective, **high throughput** scoring methodology are clearly indicated.

L4 ANSWER 58 OF 63 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 17
ACCESSION NUMBER: 95213433 EMBASE
DOCUMENT NUMBER: 1995213433
TITLE: Development of a robust flow cytometric assay for determining numbers of viable bacteria.
AUTHOR: Jepras R.I.; Carter J.; Pearson S.C.; Paul F.E.; Wilkinson M.J.
CORPORATE SOURCE: ASSBP, Brockham Park,Betchworth, Surrey RH3 7AJ, United Kingdom
SOURCE: Applied and Environmental Microbiology, (1995) 61/7 (2696-2701).
ISSN: 0099-2240 CODEN: AEMIDF
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Several fluorescent probes were evaluated as indicators of bacterial viability by **flow cytometry**. The probes monitor a number of biological factors that are altered during loss of viability. The factors include alterations in membrane permeability, monitored by using fluorogenic subsrates and fluorescent intercalating dyes such as propidium iodide, and changes in membrane potential, monitored by using fluorescent cationic and anionic potential-sensitive probes. Of the fluorescent reagents examined, the fluorescent anionic membrane potential probe bis-(1,3-dibutylbarbituric acid)trimethine oxonol [DiBAC4(3)] proved the best candidate for use as a general robust viability marker and is a promising choice for use in **high- throughput** assays. With this probe, live and dead cells within a population can be identified and counted 10 min after **sampling**. There was a dose correlation between viable counts determined by **flow cytometry** and by standard CFU assays for **samples** of untreated cells. The results indicate that **flow cytometry** is a sensitive analytical technique that can rapidly monitor physiological changes of individual microorganisms as a result of external perturbations. The membrane potential probe DiBAC4(3) provided a robust **flow cytometric** indicator for bacterial cell viability.

L4 ANSWER 60 OF 63 USPATFULL
ACCESSION NUMBER: 92:79498 USPATFULL
TITLE: Parallel pulse processing and data acquisition for speed, low error flow cytometry
[illegible]): van den Engh, Gerrit J., Livermore, CA, United States
Stokdijk, Willem, Livermore, CA, United States

PATENT ASSIGNEE(S): Regents of the University of California, Oakland, CA, United States (U.S. corporation)

	NUMBER	DATE	
PATENT INFORMATION:	US 5150313	19920922	
APPLICATION INFO.:	US 1990-508226	19900412	(7)

DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Lall, Parshotam S.
ASSISTANT EXAMINER: Auchterlonie, Thomas S.
LEGAL REPRESENTATIVE: Sartorio, Henry P.
NUMBER OF CLAIMS: 25
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 17 Drawing Figure(s); 10 Drawing Page(s)
LINE COUNT: 1013

AB A digitally synchronized parallel pulse processing and data acquisition system for a flow cytometer has multiple parallel input channels with independent pulse digitization and FIFO storage buffer. A trigger circuit controls the pulse digitization on all channels. After an event has been stored in each FIFO, a bus controller moves the oldest entry from each FIFO buffer onto a common data bus. The trigger circuit generates an ID number for each FIFO entry, which is checked by an error detection circuit. The system has high speed and low error rate.

L4 ANSWER 62 OF 63 MEDLINE DUPLICATE 18
ACCESSION NUMBER: 89270998 MEDLINE
DOCUMENT NUMBER: 89270998
TITLE: Fluorescence-based viability assay for studies of reactive drug intermediates.
AUTHOR: Leeder J S; Dosch H M; Harper P A; Lam P; Spielberg S P
CORPORATE SOURCE: Division of Clinical Pharmacology/Toxicology, Hospital for Sick Children, Toronto, Ontario, Canada.
SOURCE: ANALYTICAL BIOCHEMISTRY, (1989 Mar) 177 (2) 364-72.
Journal code: 4NK. ISSN: 0003-2697.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198909
AB Studies of drug toxicity, toxicologic structure-function relationships, screening of idiosyncratic drug reactions, and a variety of cytotoxic events and cellular functions in immunology and cell biology require the sensitive and rapid processing of often large numbers of cell **samples**. This report describes the development of a high-sensitivity, **high-throughput** viability assay based on (a) the carboxyfluorescein derivative 2'-7'-biscarboxyethyl-5(6)-carboxyfluorescein (BCECF) as a vital dye, (b) instrumentation capable of processing multiple small (less than 100 cells) **samples**, and (c) a 96-well unidirectional vacuum filtration plate. Double staining of cultured peripheral blood mononuclear cells with BCECF and propidium iodide (PI) showed no overlap between PI+ (nonviable) and BCECF+ (viable) cells by **flow cytometric** analysis. Optimal conditions were developed for dye loading and minimizing physical cell damage and fluorescence quench during the assay procedure. The ratio of BCECF fluorescence to internal standard fluorescent particles was linear from 40 to greater than 20,000 cells with a signal:noise ratio of approximately 3 at 40 cells/well. Sulfamethoxazole hydroxylamine (SMX-HA) was used as a model toxic drug metabolite to explore the validity of the BCECF procedure. SMX-HA, but not its parent compound sulfamethoxazole, resulted in a dose dependent loss of cellular fluorescence and the parallel accumulation of PI+ nonviable cells. When compared to the currently used tetrazolium dye reduction viability assay, the BCECF method was 3-fold more sensitive, greater than 10-fold faster, and required 1/10-1/100 the cell numbers.

L4 ANSWER 63 OF 63 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 83065679 EMBASE
DOCUMENT NUMBER: 1983065679
TITLE: A new flow cytometric transducer for fast **sample** throughput and time resolved kinetic studies of biological cells and other particles.
AUTHOR: Kachel V.; Glossner E.; Schneider H.
CORPORATE SOURCE: Max Planck Inst. Biochem., D-8033 Martinsried, Germany
SOURCE: Cytometry, (1982) 3/3 (202-212).
CODEN: CYTODQ
COUNTRY: United States
DOCUMENT TYPE: Journal
005 General Pathology and Pathological Anatomy
022 Human Genetics
English

AB In state of the art **flow cytometric** transducers, the cells are supplied through tubes. Passage through the tube and washing between different **samples** is time consuming and limits the number of **samples** that can be processed in a given time. This is a drawback particularly with automatic routine instruments. For kinetic studies in the time range of seconds, it is necessary to perform the cell reactions directly in the transducer in order to have a short delay between the suspension vessel where the cell reaction is in progress and the point of measurement. A new one parameter electrical sizing transducer without a particle supplying tube is described and compared with a conventional Metricell transducer. The cells are directly supplied from an exchangeable vessel to the measuring point in the transducer. The vessel which is an inexpensive mass produced product, serves as the injection tip for passing the cell suspension into the focusing **flow** path. There is no interconnected tube to delay or intermix the cells in the stage between reaction in progress in the vessel and **flow** analysis. Delay times of only 1 second are achieved with the new transducer, and by supplying each **sample** with its own vessel subsequent **samples** are analyzed without the necessity of cleaning a cell supplying tube. In this way a **high throughput** of **samples** per time unit is achieved and time kinetic experiments in the time range of seconds can be performed. The design of a tubeless multiparameter Fluvo Metricell transducer is explained.

(FILE 'HOME' ENTERED AT 11:01:50 ON 21 MAR 2001)

FILE 'MEDLINE, EMBASE, SCISEARCH, USPATFULL' ENTERED AT 11:02:03 ON 21 MAR 2001

L1 401 S (FLOW (S) CYTOMET?) AND (HIGH THROUGHPUT)
L2 140 S (FLOW (S) CYTOMET?) (10P) (HIGH THROUGHPUT)
L3 91 S L2 AND (?SAMPL?)
L4 63 DUP REM L3 (28 DUPLICATES REMOVED)